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EFFECT OF PHYTOBIOTICS (MIXTURE OF GARLIC, GINGER AND CHAYA LEAF) ON GROWTH PERFORMANCE, HAEMATOLOGICAL AND BIOCHEMICAL INDICES OF PULLET CHICKS

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ABSTRACT

The aim of this study was to investigate the effect of mixture of garlic, ginger and Chaya leaf (hereafter termed "Phytomix") at graded levels on the growth performance, haematological indices and serum biochemistry of pullet chicks fed corn-soybean-based diets. Six hundred, 1-day-old females of ISA Brown pullet chicks were assigned to five treatment groups, each group was replicated six times (20 birds in each). Birds were fed basal diet supplemented with Phytomix at different levels: 0.0, 2.5, 5.0, 7.5 and 10.0 g.kg^{-1} throughout the experimental period, which lasted for 56 days. Phytochemical screening revealed the presence of tannins, polyphenols, terpenes, saponins, flavonoids, alkaloids and cardiac glycosides in garlic, ginger and Chaya leaf, while the cyanides were found only in Chaya leaf. Highest quantities of bioactive substances were found in Chaya leaf followed by ginger and garlic. Pullet chicks, fed the diets supplemented with phytomix, had higher ($P < 0.05$) weight gain, reduced feed intake, better FCR and improved liveability when compared to the control diet. In addition, phytomix supplementation showed a linear improvement in growth performance of pullet chicks. Dietary phytomix inclusion significantly ($P < 0.05$) influenced the RBC and WBC of pullet chicks with highest values obtained in birds fed the diets containing 5.0 and 10.0 g.kg^{-1}, respectively. Inclusion of phytomix in the diets of pullet chicks produced significant ($P < 0.05$) effect on all serum chemistry parameters measured, except creatinine and AST ($P > 0.05$). It was concluded that dietary phytomix supplementation up to 10.0 g.kg^{-1} had positive effect on growth performance and health status of pullet chicks.

Key words: pullet chicks; growth; blood indices; phytobiotics; Chaya leaf

INTRODUCTION

The increasing awareness and the search for high-quality, safe animal products led to the growing interest in the use of phytobiotics as feed additives. Phytobiotics are known to exhibit antioxidant, anti-proliferate, anti-carcinogenic, anti-inflammatory and immunomodulatory, anti-diarrheic, hypolipidemic, detoxifying, digestion-stimulating, and flavoring properties (Grashorn, 2010). The bioactive substances of several plant parts (bark, leaves, stem, roots, fruits, flower, seeds) and their extracts, which are responsible for such nutritional and medicinal benefits, have been screened, and there is a wide variation in qualitative and quantitative composition of secondary metabolites both within and among such phytogenics (Kuti and Konoru, 2006).

Garlic (*Allium sativum* L.) of Alliaceae family has been reported to contain bioactive constituents

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such as alliin, ajoene, diallylsulfide, flavonoids, saponins, enzymes (allinase), B-vitamins and minerals (Suleria *et al.*, 2015). Ginger (*Zingiber officinale* Roscoe.), of Zingiberacea family contains active ingredients including essential oils (zingiberene, zingiberol, D-camphor), Shogaols, Diarylheptanoids, Gingerols, Paradol, Zerumbone, 1-Dehydro-(10) gingerdione, Terpenoids and Ginger flavonoids, which are responsible for its pungency and anti-serotonin effect, for enhanced gastrointestinal function and mucin synthesis, as well as other biological activities (Ganguly, 2017). Chaya leaf (*Cnidoscolus aconitifolius* (Mill.) Johnston), of Euphorbiaceae family, has good record of being used ethno-medically for prophylaxis and curative treatment of ailments such as diabetes, obesity, kidney stones, insomnia, gout, hemorrhoids, scorpion stings, acne and the eye problems. Its efficacy as anti-diuretic, laxative, appetite and digestion-stimulant has been proven (Jensen, 1997; Donkoh *et al.*, 1999). However, only few studies quantified the phytochemicals presence in these selected phytobiotics, thus the need for this study.

Studies on the use of garlic and ginger, solely or as a mixture, as feed additives in poultry feeding have been well-documented. Elagib *et al.* (2013) reported that 3 % dietary garlic supplementation resulted in increased feed intake, higher body gain and better feed utilization. Ademola *et al.* (2009) also observed higher body gains and improved FCR in broiler chickens fed garlic and ginger mixture. Ademola *et al.* (2012) reported that mixture of ginger and garlic produced positive effects on growth performance of pullet growers, hen-day production and egg weight of layers, whereas Bamidele and Adejumo (2012) reported non-significant effect on growth performance of pullet growers fed diets containing mixture of ginger and garlic.

However, there is a paucity of available literature on the combined use of garlic, ginger and chaya as feed additives for pullet chicks. In this study, we hypothesized that synergistic effects of bioactive ingredients present in garlic, ginger and Chaya leaf will enhance growth performance and improve health status of pullet chicks. This study, therefore, attempts to quantify the phytochemical constituents of garlic, ginger and Chaya leaf, and also assess the response of pullet chicks to graded levels of Phytomix (garlic, ginger and Chaya leaf mixture) in terms of growth, haematology and serum biochemical indices.

MATERIALS AND METHODS

Ethical Approval

The study protocol and procedures were conducted in accordance with the Animal Care and Use Review Committee guidelines of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Nigeria.

Location of the Study

This trial was conducted at the Poultry Farms and Agricultural Science Laboratory of the Department of Agricultural Education, School of Vocational Education, Federal College of Education, Abeokuta, Ogun State, Nigeria.

Collection and Preparation of Phytobiotics

Garlic bulbs and ginger rhizomes used in this study were grown in Nasarawa State of Nigeria due to the prevailing favourable growing conditions. Garlic bulbs (Soft neck variety) were harvested at maturity (5 months), while ginger rhizomes (UG 1 variety) were harvested at full maturity of 9 months. Thereafter, they were transported to Abeokuta, Ogun State. Fresh Chaya leaf samples (*C. aconitifolius*) were harvested from 2-years-old Chaya plants, which were planted by stem cuttings, at the Crop Production Farm near Osiele village in Abeokuta. They were identified at the Department of Pure and Applied Botany, Federal University of Agriculture, Abeokuta, Nigeria. Garlic bulbs were separated into cloves and peeled. Ginger rhizomes were cleaned and manually chopped to air drying. Chaya leaves were thoroughly washed with clean water and chopped to 2-3 cm in size to air drying. Garlic, ginger and Chaya leaf samples were air dried at temperature between 25.50 – 28.30 °C (Mean ± SD; 26.58 ± 1.02 °C) for 2-3 weeks until the desired level of dryness was obtained. Thereafter, they were milled individually to powder and stored in air-tight bags until phytochemical analysis and incorporation into the formulated diet.

Phytochemical Screening

Phytochemical screening for major constituents was performed using standard qualitative procedures as previously described (Trease and Evans, 1989; Mordi and Akanji, 2012). The test for tannins was carried out by dissolving 0.5 g of the dried powdered plant extract in 20 mL of distilled water, then filtered and 0.1 % ferric chloride reagent was added to the filtrate. For cardiac glycosides, 0.5 g of extract was added to 2 mL of acetic anhydrate plus H_2SO_4 using killer kiliani test. The test for alkaloids was carried out by adding 0.5 g of an aqueous extract into 5 mL of 1 % HCl, boiled and filtered. Then Mayer's reagent was added. The extract was subjected to frothing test for the identification of saponin. Haemolysis test was then performed on the frothed extracts in water to remove false positive results. The extract was also tested for free glycoside-bound anthraquinones by adding of 5 g of the extract to 10 mL of benzene, then filtered and ammonia solution was added. The presence of flavonoids was determined using 1 % aluminum chloride solution in methanol-concentrated HCL, magnesium turnings and potassium hydroxide solution.

Management of Birds and Diets

A total of six hundred (600) 1-day-old female, ISA Brown pullet chicks were purchased from a reputable hatchery in Abeokuta, Nigeria. They were randomly allocated on weight equalization basis to five dietary groups, each having six replicates with 20 birds per replicate. Basal diet (Corn-soybean based) was formulated to meet the nutrient requirement of pullet chicks (Table 1). Proximate analysis of basal diet (crude protein, crude fibre, ether extract and ash) was carried out using the standard procedure of the Association of Official Analytical Chemists (AOAC, 1995). Selected phytobiotics (garlic, ginger and Chaya leaf) were mixed in equal proportion on weight-for-weight basis to form "Phytomix" and the graded levels

Table 1. Composition of experimental basal diet for pullet chicks (0 – 8 weeks)

Ingredients	Composition ($g.kg^{-1}$)
Maize	520.00
Soybean meal (45% CP)	270.00
Palm kernel cake (PKC)	60.00
Groundnut cake (GNC)	20.00
Wheat offal	71.50
Bone meal	20.00
Limestone	30.00
L-Lysine HCl	1.20
DL-Methionine	1.80
Vitamin/Mineral Premixa	2.50
Industrial Salt (NaCl)	2.50
Enzyme (Fullzyme®)[b]	0.25
Toxin binder (Zerotox®)[b]	0.25
Total	1000
Determined analysis ($g.kg^{-1}$, except ME)[c]	
Metabolizable Energy ($kcal.kg^{-1}$)[d]	2785.50
Crude protein	205.30
Ether extract	37.20
Crude fibre	41.60
Total ash	295.00

[a]Provided vitamin-mineral premix per 2.5 kilogram of diet: 12,000,000 IU vitamin A; 2,500,000 IU vitamin D_3; 30,000 IU vitamin E; 2,000 mg vitamin K_3; 2,250 mg vitamin B_1; 6,000 mg vitamin B_2; 4,500 mg vitamin B_6; 15 mcg vitamin B_{12}; 40,000 mg niacin; 1,500 mg folic acid; 50 mcg biotin; 15,000 mg pantothenic acid; 300,000 mg choline chloride; 125,000 mg antioxidant (butylhydroxytoluene); 80,000 mg Mn; 50,000 mg Zn; 5,000 mg Cu; 20,000 mg Fe; 1,000 mg I; 200 mg Se; 500 mg Co; Produced by Rotinol International Ltd., Nigeria; [b]Manufactured by Biofeed Technology Inc., Canada, [c]Mean values of 3 replicates; [d]ME was estimated from the proximate values

of 0.0, 2.5, 5.0, 7.5 and 10.0 g.kg^{-1} were included in the basal diet to form five experimental diets. Birds were fed one of five diets for the experimental period of 56 days. The birds had unrestricted access to feed offered in mash form during the study. Biosecurity measures and vaccination programme were strictly adhered to.

Data Collection and Analysis

Growth Performance

Bodyweight of the birds per replicate was measured on a weekly basis in order to compute weight gain. Daily feed intake was also measured as the difference between the feed offered and leftovers in order to estimate feed conversion ratio. A record of mortality was kept as it occurred. Liveability was computed as proportion of live birds left after the experiment.

Haematology and Serum Biochemistry Indices

At 56th day, blood samples (2.5 ml each) were collected from two birds in each replicate (*n = 12* per treatment) with needle and syringe through the jugular vein directly into EDTA-containing bottles and plain bottle for the determination of haematological indices and serum chemistry, respectively, using standard procedures, as described by Weiss and Wardrop (2010) and Fafiolu *et al.* (2014).

Statistical Analysis

The generated data were subjected to a One-way Analysis of Variance (ANOVA) in a Completely Randomized Design using General Linear Model procedure of SAS for Windows 9.1.3 version (SAS, 2007). Significant means among the treatments were compared according to Tukey's HSD test at 95 % probability level. Orthogonal polynomials were used to assess the linear and quadratic effects of varying levels of garlic, ginger and Chaya leaf mixture.

RESULTS AND DISCUSSION

Phytochemical Analysis of Garlic, Ginger and Chaya Leaf

Phytochemical screening revealed presence of tannins, polyphenols, terpenes, saponins, flavonoids, alkaloids and cardiac glycosides in garlic, ginger and Chaya leaf, while cyanides were found only in Chaya leaf. Highest quantities of bioactive substances were found in Chaya leaf, followed by ginger and garlic (Table 2). This suggests that Chaya leaf holds a potential to offer better nutritional and health benefits as feed additive and functional food compared to garlic and ginger. Tannins are known to promote the healing of wounds and burns and also to possess anti-diarrhoeal and anti-haemorrhagic agent (Mordi and Akanji, 2012). Presence of polyphenols and terpenes would ensure better oxidative stability by scavenging the reactive oxygen species (ROS) and free radicals that could damage DNA of cells, thereby regulating enzyme systems that play crucial role in the *in vivo* redox homeostasis at the cellular level (Muthusamy and Sankar, 2015). Also, this probably suggests that there could be a delayed lipid peroxidation process in fresh and processed poultry products obtained from birds fed diets containing these bioactive substances (Botsoglou *et al.*, 2003). Phenols have

Table 2. Phytochemical analysis of garlic, ginger, and Chaya leaf[#]

Items (mg.100 g^{-1})	Garlic	Ginger	Chaya leaf
Tannins	0.90	1.70	7.80
Polyphenols	69.13	118.04	184.07
Terpenes	1.80	3.90	4.60
Saponins	86.15	157.05	321.00
Flavonoids	2.10	3.30	3.80
Alkaloids	128.11	327.00	526.01
Glycosides	39.00	112.85	126.72
Cyanides	0.00	0.00	0.596

[#]Mean values on 3 replicates

been noted to moderate enzymatic activities that cause inflammation, thereby suggesting its anti-inflammatory properties (Muthusamy and Sankar, 2015).

Saponins helps to fight parasitic infections in plants, and supports the immune system to confer antimicrobial protection against viruses and bacteria in man and animals (Tende *et al.*, 2014). It also plays an active role in the urea metabolism by lowering the activity of the intestinal and faecal urease, thus, causing reduced NH_3 formation, thereby enhancing kidney formation. Moreover, saponins exert haemolytic, hypocholesterol and cardiac depressant properties, thus, implying healthy cardiac function and reduced risk of cardio-vascular diseases (Muthusamy and Sankar, 2015). The steroidal saponins, alongside with organosulfur constituents in garlic, are direct antioxidants and show cholesterol-lowering effects, which may result in reduced risk of coronary and cardiovascular diseases. Tende *et al.* (2014) reported that anti-hypercholesterol effects improved serum lipid profile in Wistar rats, as well as cardio-protective effect on isolated perfused (rabbit) heart, caused by single and combined doses of administered ginger and garlic. These effects could be attributed to the inherent organosulfur constituents and steroidal saponins. Flavonoids, due to their high anti-oxidative activities, were known to exert anti-allergic, anti-inflammatory, anti-microbial, anti-proliferative and anti-carcinogenic effects, while alkaloids have been noted for their detoxifying, analgesic, anti-spasmodic, anti-bacterial and hypotensive properties (Akachukwu *et al.* 2014).

Relative variation in quantitative values of phytochemical constituents obtained in this study, compared to those reported by Akachukwu *et al.* (2014), could be attributed partly to the differences in plant growing conditions, age at harvest, processing methods (air-dried vs oven-dried), and extraction methods. Moreover, cyanide content determined in chaya leaf (5.96 $mg.kg^{-1}$) will probably not produce any chronic health disorder in pullet chicks because of its lower concentration when compared to the recommended safe cyanide level (10 mg HCN equivalents/g fresh weight) in food products containing cyanogens (FAO/ WHO, 1991) and its dietary inclusion level.

Growth Performance

Influence of diets supplemented with phytomix on growth performance of pullet chicks is shown in Table 3. Final weight and daily weight gain increased progressively ($P < 0.05$) with increase in phytomix level (linear, $P = 0.014$). Feed intake reduced ($P < 0.05$) as phytomix supplementation increased, however, the least value was observed in birds fed the diet containing 5.0 $g.kg^{-1}$ phytomix (linear, $P = 0.003$). Feed conversion ratio (FCR) of pullet chicks decreased significantly ($P = 0.0049$) as phytomix supplementation increased from 0 to 10.0 $g.kg^{-1}$ (linear, $P < 0.001$). Phytomix supplementation had positive effect ($P < 0.05$) on liveability of pullet chicks as no mortality was recorded in birds fed the diet supplemented with 10.0 $g.kg^{-1}$ phytomix. The improvement in body gain of pullet chicks fed phytomix could be due to its positive effect on palatability and digestion stimulation by endogenous

Table 3. Growth performance of pullet chicks (0 – 8 weeks) fed diet supplemented with phytobiotics (mixture of chaya leaf, garlic and ginger powder)[#]

Items	Level of phytobiotics inclusion ($g.kg^{-1}$)					Pooled	Significance		
	0	2.5	5.0	7.5	10.0	SEM	*P*-value	Linear	Quadratic
Initial weight (g)	34.40	34.43	34.50	34.10	34.13	0.126	0.7845	0.815	0.799
Final weight (g)	379.33^{b}	379.41^{b}	389.69^{ab}	420.59^{a}	434.21^{a}	7.748	0.0139	0.014	0.734
Daily weight gain (g)	6.16^{c}	6.16^{c}	6.34^{bc}	6.90^{b}	7.14^{a}	0.139	0.0365	0.014	0.738
Daily feed intake (g)	44.01^{a}	42.05^{ab}	38.84^{b}	41.43^{ab}	39.41^{ab}	0.629	0.0287	0.003	0.434
FCR	7.16^{a}	6.86^{ab}	6.12^{bc}	6.01^{bc}	5.52^{c}	0.184	0.0049	<0.001	0.723
Livability (%)	93.33^{c}	95.00^{bc}	95.00^{bc}	98.33^{ab}	100.00^{a}	0.766	0.0067	0.001	0.418

[#]Mean values on 6 replicate pen, Values in the same row having different superscripts differ @ $P < 0.05$, FCR = Feed conversion ratio, SEM = standard error of the mean enzyme secretion, which may enhance nutrient role for development of the digestive system, muscle tissue accretion, immune system and to improve the growth performance. Ginger is known to contain a protein digesting enzyme (zingibain), which is supposed to improve digestion. Moreover, dietary inclusion of ginger into animal diets caused notable increase in the amount of pancreatic and intestine lipase (Ganguly, 2017). According to Muthusamy and Sankar (2015), phytobiotics could also increase the output of digestive enzymes from the pancreas, gut mucosa and increased bile flow for improved nutrient metabolism.

Reduced (P < 0.05) feed intake and improved FCR in birds fed phytomix is an indication of better feed utilization efficiency. Chick phase (0-6 weeks) is a critical period for development of digestive tract organs and the immune system. Efforts to deliver the rightful needed dietary nutrients and substrates to enhance biosynthesis of intestinal epithelial cells and to boost cell-mediated and humoral immune system will go along to reduce birds' susceptibility to infectious diseases, physiologic and environmental stress throughout their lifetime. These findings indicate that the level of inclusion and synergistic interaction between phytochemical constituents of phytobiotics determines their resultant efficacy in birds. These results are in concert with the data of Ademola *et al.*, (2012), who reported that mixture of ginger and garlic induced positive effects on growth performance of pullet growers, hen-day production and egg weight of layers. In addition, Ademola *et al.* (2009) and Oleforuh-Okoleh *et al.* (2015) observed higher body gains and improved FCR in broiler chickens fed garlic and ginger mixture. Elagib *et al.* (2013) also noted that dietary garlic supplementation led to the higher body gain and best feed utilization efficiency in broiler chickens. Liveability was positively (P < 0.05) influenced by the treatments. This is in agreement with Donkoh *et al.*, (1999), who reported decreased mortality rate in broiler chickens fed the diet containing chaya leaf meal (CLM) in two trials. Decrease in mortality rate with increased phytomix addition confirmed their antioxidant, anti-inflammatory and anti-microbial properties. Garlic, ginger and Chaya leaf are known to contain appreciable amount of vitamin C, a potent antioxidant, which prevents or reduces cellular damage. The metabolic activities of allicin and other organosulfur compounds, present in garlic, inhibit the activity of inflammatory enzymes in the body, have greater antioxidant potential and enhance the activity of thymus gland to stimulate the proliferation of the body T-cells and interleukin-2 (IL-2), which plays active role in the immune system function (Suleria *et al.*, 2015). Ganguly (2017) had earlier reported that ginger is able to regulate the activities of antioxidant enzymes, such as superoxide dismutase, catalase and glutathione peroxidase, thereby prevent or delay lipid peroxidation. Therefore, the synergy of bioactive

Table 4. The haematological indices of pullet chicks (0 – 8 weeks) fed diet supplemented with phytobiotics (mixture of chaya leaf, garlic and ginger powder)#

Items	Level of phytobiotics inclusion ($g.kg^{-1}$)					Pooled	Significance		
	0	2.5	5.0	7.5	10.0	SEM	*P*-value	Linear	Quadratic
PCV (%)	25.50	25.50	28.00	24.50	26.50	0.431	0.0773	1.000	0.513
Hb ($g.100\ ml^{-1}$)	8.55	8.60	9.45	8.00	8.80	0.165	0.0575	0.668	0.933
RBC (x $10^{9/L}$)	2.09^{b}	2.28^{ab}	2.69^{a}	2.34^{ab}	2.43^{ab}	0.067	0.0331	0.984	0.180
WBC (x $10^{12/L}$)	9.25^{b}	9.25^{b}	11.00^{ab}	10.00^{ab}	12.25^{a}	0.372	0.0146	0.104	0.002
Neutrophils (%)	12.00	17.50	21.00	17.50	18.50	1.429	0.4148	0.234	0.189
Lymphocytes (%)	86.00	80.50	76.50	81.00	78.00	1.472	0.3240	0.155	0.262
Monocytes (%)	1.00	1.50	1.00	0.00	1.50	0.272	0.4413	0.867	0.485
Eosinophils (%)	1.00	0.50	1.00	1.50	1.50	0.235	0.7019	0.175	0.551
Basophils (%)	0.00	0.00	0.50	0.00	0.00	0.072	0.0723	1.000	0.260

#Mean values on 12 birds/treatment, Values in the same row having different superscripts differ @ P < 0.05, SEM = standard error of the mean substances in garlic, ginger and chaya may support the chicks' protective mechanisms against oxidative damage and enhance their immune system, thereby suppressing their susceptibility to infections.

Haematology

Dietary phytomix inclusion significantly ($P < 0.05$) influenced the RBC (red blood cells) and WBC (white blood cells) of pullet chicks with highest values obtained in birds fed the diets containing 5.0 and 10.0 g.kg^{-1}, respectively (Table 4). In addition, WBC of pullet chicks increased quadratically ($P = 0.002$) as phytomix supplementation increased. Increased RBC implies that dietary phytomix enhanced haematopoietic process, morphology and osmotic fragility of erythrocytes produced with no any adverse effect on physiological and metabolic functions of the body. This confirms the ability of the phytochemical constituents present in garlic, ginger and chaya to improve the health status of the pullet chicks. Donkoh *et al*., (1999) reported increased RBC in broiler chickens fed diets containing chaya leaf meal (CLM). Tende *et al*., (2014) reported that RBC activates the signalling of cardio-protective process by converting the organosulfur compounds in garlic to hydrogen sulphide (H_2S), an endogenous cardio-protective vascular cell signalling molecule, thus stimulating blood circulation. Also, ginger extract was found to improve blood flow in animals due to significant (dose-dependent) decrease in the arterial blood pressure and cardio-depressant effect on the rate and force of spontaneous contractions (Tende *et al*., 2014; Suleria *et al*., 2015). Improved blood circulation will not only increase oxygen carrying capacity of the cells for better nutrient transport throughout the body system, but will also prevent fat deposition in the arteries, thereby reducing the chance of cardio-vascular diseases.

Relative higher WBC counts obtained in birds fed the diets containing phytomix indicates enhanced host's immune system by stimulating antibody production as well as creation of the unfavourable environment for pathogenic microbes thereby confirming their anti-microbial properties. This confirms the immuno-modulatory properties of garlic, ginger and chaya leaf, which is an indication, that the birds could perform their phagocytic functions for optimum immunity levels and higher disease resistance (Fafiolu *et al*., 2014). In agreement, Oleforuh-Okoleh *et al*. (2015) reported that aqueous extract of garlic and ginger induced positive effect on PCV (packed cell volume) and WBC of broiler chickens. Non-significant ($P < 0.05$) effect of phytomix on cell counts indicates no signs of lymphopenia, heterophilia and leukocytosis.

Table 5. Serum biochemical indices of pullet chicks (0 – 8 weeks) fed diet supplemented with phytobiotics (mixture of chaya leaf, garlic and ginger powder)[#]

Items	Level of phytobiotics inclusion (g.kg^{-1})					Pooled	Significance		
	0	2.5	5.0	7.5	10.0	SEM	*P*-value	Linear	Quadratic
Glucose (mg.100 ml^{-1})	148.25^{c}	156.25b^{c}	185.80^{a}	175.50^{ab}	186.75^{a}	4.640	0.0012	<0.001	0.133
Total protein (g.L^{-1})	56.70^{cd}	53.55^{d}	64.80^{ab}	69.75^{a}	61.05^{bc}	1.628	<0.0001	<0.001	0.009
Albumin (g.L^{-1})	39.20^{b}	36.70^{b}	37.95^{b}	46.35^{a}	41.30^{ab}	0.990	0.0006	0.002	0.680
Globulin (g.L^{-1})	17.50^{c}	16.85^{c}	26.85^{a}	23.40^{ab}	19.75^{bc}	1.089	0.0004	0.020	0.001
A:G	2.25^{a}	2.24^{a}	1.43^{b}	1.98^{ab}	2.11^{a}	0.098	0.0119	0.249	0.115
Urea (mg.100 ml^{-1})	7.50^{a}	7.40^{a}	6.90^{a}	5.40^{ab}	4.25^{b}	0.392	0.0039	0.833	0.026
Creatinine (mg.100 ml^{-1})	0.55	0.55	0.65	0.55	0.50	0.016	0.1245	0.153	0.153
AST (IU.L^{-1})	98.65	108.30	113.80	126.65	114.90	4.172	0.3336	0.102	0.317
ALT (IU.L^{-1})	12.40^{c}	17.60^{b}	13.65^{c}	12.80^{c}	22.65^{a}	1.047	<0.0001	<0.001	<0.001
AST/ALT	7.95^{ab}	6.15^{b}	8.56^{ab}	9.90^{a}	5.08^{b}	0.544	0.0086	0.514	0.031

[#]Mean values on 12 birds/treatment, Values in the same row having different superscripts differ @ $P < 0.05$, A:G = Albumin/globulin ratio, SEM = standard error of the mean.

Serum Biochemistry

Serum biochemical indices of pullet chicks fed the diets supplemented with phytomix are shown in Table 5. Inclusion of phytomix into the diets of pullet chicks produced significant ($P < 0.05$) effect on all serum biochemistry parameters measured, except ($P > 0.05$) creatinine and AST. Serum glucose increased linearly ($P < 0.001$) as phytomix inclusion increased from 0 to 10.0 g.kg^{-1}. The highest total protein (linear, $P < 0.001$; quadratic, $P = 0.009$) was obtained in birds fed the diets containing 7.5 g.kg^{-1}. In a similar manner, the highest albumin (linear, $P = 0.002$) was obtained in birds fed the diets containing 7.5 g.kg^{-1}, while the highest globulin value (linear, $P = 0.020$; quadratic, $P = 0.001$) was obtained in birds fed the diets containing 5.0 g.kg^{-1}.

Higher serum glucose level may indicate enhanced energy metabolism due to stimulation of endogenous digestive enzymes by phytomix and due to the release of adequate substrate (glucose) needed for mechanical work and body maintenance. This could be attributed to higher weight gain observed in chicks fed the diets containing 10.0 g.kg^{-1} phytomix.

Assessment of serum proteins in poultry birds is important because they play crucial role in the maintenance of colloid osmotic pressure, mobility of dietary nutrients, minerals and hormones, as a rapid substitute for indispensable amino acids, as well as biosynthesis of enzymes and immune system (Piotrowska *et al*. 2011). Albumin carries and delivers important nutrients to body cells. It is important for tissue growth and healing as it supplies appropriate amino acids needed for tissue proteins synthesis in the period of quick somatic growth of birds (Piotrowska *et al*. 2011), and also binds to toxins and free radicals, thus minimizing oxidation process and preventing cell damage. In this present study, the feeding with a phytomix above 5 g.kg^{-1} produced higher albumin in birds indicating that such higher concentration of garlic, ginger and chaya leaf would be required to exert their wound healing and antioxidant effect. Also, higher total protein and albumin could be a pointer to enhanced liver functions by phytomix, since plasma proteins are synthesized predominantly by the liver. Higher globulin, observed in birds fed 5 g.kg^{-1} phytomix and above, implies improved host's immune system and could be attributed to reduced mortality observed in birds fed such diets. The present finding is strongly supported by Oleforuh-Okoleh *et al*. (2015), who reported significant increase in serum total protein, albumin and globulin of broiler chickens fed aqueous extract of garlic and ginger.

Serum urea concentration reduced ($P < 0.05$) significantly (quadratic, $P = 0.026$) as phytomix level increased. Urea concentration provides insight to diagnosis of renal damage or functionality of the kidney. Reduced ($P < 0.05$) serum urea concentration in pullet chicks confirms that the selected phytobiotics exhibited digestive-stimulating effect, which translated to better digestion, absorption and utilization of protein in dietary treatment groups, which invariably led to improved protein utilization without any harmful effect on the functionality of liver and kidney.

Higher ALT value (linear, $P < 0.001$; quadratic, $P < 0.001$) was recorded in chicks fed the diet containing 10.0 g.kg^{-1} phytomix. Alanine aminotransferase (ALT) is a liver enzyme that catalyzes the transfer of amino groups from L-alanine to α–ketoglutarate in order to produce hepatic metabolite oxaloacetate (Kim *et al*., 2008). ALT also plays an important role in biotransformation and detoxification of various toxicants, ROS, endo- and xerobiotics. Higher ALT values in birds fed 10 g.kg^{-1} phytomix could be attributed to higher serum glucose level. Kim *et al*. (2008) had reported a positive correlation between serum glucose level and the ALT activity. Kim *et al*. (2008) noted that the serum ALT activity may be affected by a number of factors that are not associated with hepatic necrosis, and that the elevated ALT values, which are the five-times less than the upper limit of the normal range, non-persistent, and without any clinical symptoms, may not be worrisome. Therefore, the synergistic effect of bioactive substances in selected phytobiotics improved the health status of pullet chicks.

CONCLUSION

Phytochemical analysis showed that highest quantities of bioactive substances (tannins, polyphenols, terpenes, saponins, flavonoids, alkaloids, and cardiac glycosides) were found in Chaya leaf followed by the ginger and garlic. Pullet chicks fed diets, supplemented with phytomix, had significantly ($P < 0.05$) higher weight gain, reduced feed intake, better FCR and improved liveability when compared with the control diet. In addition, phytomix supplementation showed a linear improvement in growth performance of pullet chicks. Dietary phytomix supplementation, in this study, led to the increased RBC and WBC formation. Phytomix inclusion resulted in higher concentration of serum glucose, total protein, albumin and globulin, as well as reduced serum urea concentration in pullet chicks.

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